**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO: 33076**

**Manuscript Type:** ORIGINAL ARTICLE

**Title: Systemic interleukin-9 in IBD: association with mucosal healing in ulcerative colitis**

**Authors: Malgorzata Matusiewicz, Katarzyna Neubauer, Iwona Bednarz-Misa, Sabina Gorska, Malgorzata Krzystek-Korpacka**

**Correspondence to:** Malgorzata Krzystek-Korpacka, PhD, DSc (Med), Dept. of Medical Biochemistry, Wroclaw Medical University, ul. Chalubinskiego 10, 50-368 Wroclaw, Poland. E-mail: malgorzata.krzystek-korpacka@umed.wroc.pl; gosia.krzystek@gmail.com

Tel: +48 71 784 13 95

Fax: + 48 71 784 00 85.

*1. What did this study explore?*

This study explores how circulating IL9 differs in patients with inflammatory bowel disease as compared to healthy individuals, how its levels are related to the disease activity, both clinical and endoscopic, as well as to the disease-associated anemia and cachexia. It also assesses the potential of circulating IL9 as a mucosal healing marker.

*2. How did the authors perform all experiments?*

Blood samples were obtained from study participants and the concentrations of IL9 as well as other cytokines were measured in their sera by means of flow cytometry-based method using Luminex xMAP® technology and Bio-Plex Pro™ Human Cytokine, Chemokine, and Growth Factor Magnetic Bead–Based Assays on Bio-Plex 200 platform with HRF (Bio-Rad, USA). High-sensitive C-reactive protein was measured using latex particle-enhanced immunoturbidimetric method with the CRPex-HS CRP test (Good Biotech Corp., Taichung, Taiwan) and with protein multicalibrator (ProDia International, Sharjah, UAE). Data for other parameters were retrieved from patients' history.

*3 How did the authors process all experimental data?*

The fluorescence recorded for analyzed samples, the intensity of which was proportional to the concentrations of IL9, was read against standard curves drawn for cytokine standards using 5-PL logistic regression and the data were analyzed using BioPlex Manager 6.0 software. The obtained concentrations were subjected to statistical analysis using MedCalc Statistical Software version 16.8.4 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015) including testing for normality of distribution and homogeneity of variation, the analyses of variance (one and two-factor), correlation, and frequency, logistic and multiple regression, and Receiver Operating Characteristics (ROC) analysis.

*4 How did the authors deal with the pre-study hypothesis?*

Taking into account strong association between mucosal healing and leukocyte expression of IL9 (mRNA) reported by others, we hypothesized that circulating IL9 may also be associated with mucosal healing and act as a surrogate marker. Its evaluation is faster, simpler and less expensive than mRNA analysis. First, to test this hypothesis we related IL9 concentrations to Mayo endoscopic score reflecting severity of bowel inflammation. Upon finding a strong correlation, we subsequently evaluated IL9 accuracy as a marker of mucosal non-healing by means of ROC analysis.

Second hypothesis was that since IL9 was implicated in induction of weight loss in animal model of colitis, IBD patients with cachexia would have more pronouncedly elevated levels of IL9. To address this hypothesis we stratified our IBD patients into those who recently reported substantial involuntary weight loss and those who retained their normal weight and compared IL9 levels in both groups. We also sought correlation between IL9 and hemoglobin concentrations as cachexia is frequently accompanied by anemia.

The others observed that tissue overexpression of IL9 has been predominantly associated with UC but it has not been confirmed on systemic level. However, the study on systemic IL9 has been conducted on a small cohort of IBD patients. Therefore, our third hypothesis was that substantial enlargement of the cohort of IBD

patients would permit the clear manifestation of the suggested tendencies. If not, we hypothesized that there might be at least some differences in the pattern of association with the other cytokines, a phenomenon we observed earlier. To verify the hypothesis we analyzed IL9 in 171 IBD patients of whom 97 had CD and 74 had UC. Since there was no significant difference in IL9 between CD and UC, either active or inactive, we analyzed the pattern of IL9 correlations with representatives of inflammatory cytokines (IL1β, IL6 and TNFα), angiogenic factors (VEGF-A) and Th1 cytokines (IFNγ) and Th2 cytokines (IL13).

*5 What are the novel findings of this study?*

This study shows for the first time that the elevation of IL9 is more pronounced in IBD patients who suffered from substantial and involuntary weight loss and that cytokine concentrations inversely correspond with hemoglobin levels, linking IL9 elevation with cachexia and anemia of chronic diseases. It also shows that an elevation of systemic IL9 in ulcerative colitis corresponds with mucosal inflammation and that IL9 displays high accuracy as a negative marker of mucosal healing. Moreover, our results show differences in the interplay between IL9 and other cytokines between ulcerative colitis and Crohn's disease. IL9 seems to be more tightly associated with proinflammatory and Th1 cytokines in ulcerative colitis and with angiogenic and Th2 cytokines in Crohn's disease.

Małgorzata Mizia-Stec